Genetic variation in interleukin 8 and its receptor genes and its influence on the risk and prognosis of prostate cancer among Finnish men in a large cancer prevention trial

Hannah P. Yang[a], Karen Woodson[b], Philip R. Taylor[b], Pirjo Pietinen[c], Demetrius Albanes[d], Jarmo Virtamo[c] and Joseph A. Tangrea[b]

The cytokine interleukin 8 (IL-8) may play a role in the pathogenesis of prostate cancer through the modulation of tumour immune response or enhanced angiogenesis. A common polymorphism of the IL-8 (−251) gene, which may affect the production level of the cytokine, has been inversely associated with a number of diseases, including prostate cancer. We examined the most representative single nucleotide polymorphisms (SNPs) for the IL-8 and its receptors (CXCR1 and CXCR2) genes, and conducted a case–control study nested within the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study to examine if these SNPs are associated with susceptibility to and prognosis of prostate cancer. Using incidence density sampling, 584 cases of primary prostate cancer and 584 matched controls were selected. In this population, we observed no strong association between the SNPs for IL-8 −251 (A→T), CXCR1 +860 (C→G) and CXCR2 −1010 (A→G) and either the subsequent risk of prostate cancer or individual prognostic factors among cases. Although none of the SNPs studied are likely to have major effects on prostate cancer susceptibility, a role for other polymorphisms associated within these genes cannot be excluded. ***European Journal of Cancer Prevention*** **15:249–253 © 2006 Lippincott Williams & Wilkins.**

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[a]Emerging Leaders Program, DHHS, Washington, DC, [b]Cancer Prevention Studies Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA, [c]Department of Epidemiology and Health Promotion, National Public Health Institute, Helsinki, Finland and [d]Nutritional Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

Correspondence to Joseph Tangrea, Program Director, Prostate and Urologic Cancer Research Group, Division of Cancer Prevention, NCI, NIH EPN/2102, 6130 Executive Blvd, Rockville, MD 20852, USA
Tel: +1 301 594 2935; fax: +1 301 435 1564;
e-mail: tangreaj@mail.nih.gov

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Introduction

A crucial step in the continuous growth of tumours and development of metastases is the recruitment of new blood vessels within and around tumours (Coussens and Werb, 2002). Interleukin 8 (IL-8), an important chemokine in the human inflammatory process has been shown to be involved in human carcinogenesis through its potential functions as an angiogenic factor, and has been implicated in a number of diseases including prostate cancer (Xie, 2001). Though one study could not document the expression of IL-8 by LNCaP cells (Balbay *et al.*, 1999), IL-8 expression has been found in several other established prostate cancer cell lines (Greene *et al.*, 1997; Moore *et al.*, 1999; Kim *et al.*, 2001; Konig *et al.*, 2004). Clinically, elevated levels of IL-8 have been reported in the serum (Veltri *et al.*, 1999) and tumour tissue specimens of prostate cancer patients (Greene *et al.*, 1997; Ferrer *et al.*, 1998; Veltri *et al.*, 1999; Konig *et al.*, 2004). Prostate cancer specimens stained positively for IL-8 whereas benign prostatic hyperplasia and normal tissue exhibited little staining (Ferrer *et al.*, 1998). Transfection of sense and antisense constructs of IL-8 using xenograft and cell line models (Inoue *et al.*, 2000), strongly suggests that IL-8 is a progression factor for prostate cancer independent of other angiogenesis factors.

IL-8 exerts its effects through two receptors: CXCR1 (previously called IL-8RA) and CXCR2 (or IL-8RB). The IL-8 receptors have also received attention because of the findings that they have an important role in angiogenesis and tumour progression (Rossi and Zlotnik, 2000). RNA transcripts for CXCR1 and CXCR2 have been detected in PC3 cells and neutralizing antibodies to CXCR2 inhibited IL-8 stimulation of cell adhesion as well as migration (Reiland *et al.*, 1999), suggesting that IL-8 can promote prostate cancer progression and metastasis through its receptor.

The relationship between IL-8 and prostate cancer makes the genes encoding for IL-8 strong candidate genes for categorizing risk and prognosticating the severity of prostate cancer. A common polymorphism of IL-8 gene on position –251 has been investigated in a

wide variety of diseases. The IL-8 TT genotype, viewed as the low producer of IL-8 (Hull *et al.*, 2000), has been shown to confer a level of protection against the onset of diseases such as Kaposi's sarcoma (van der Kuyl *et al.*, 2004), colorectal cancer (Landi *et al.*, 2003), and gastric cardia cancer (Savage *et al.*, 2004). In a recent observational study, the risk of prostate cancer was reported to be significantly lower among those with the IL-8 TT genotype [odds ratio (OR) 0.66, 95% confidence interval (CI) 0.44–0.99] (McCarron *et al.*, 2002). Thus far, this association of the genetic susceptibility of IL-8 and prostate cancer has not been replicated.

In order to further examine the genetic variation in IL-8 and its association with prostate cancer risk and progression, we conducted a case–control study nested within the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study to examine the *a priori* hypothesis that IL-8 TT genotype is associated with decreased risk of prostate cancer. In addition, we examined the effect of the genetic variation of IL-8 receptors, CXCR1 and CXCR2, on prostate cancer risk and prognosis.

Design and methods

Study population

This study was conducted in a cohort of the 29 133 white male smokers between 50 and 69 years old at entry who participated in the ATBC Study conducted in Finland (1985–1993). The overall design, rationale, objectives and main findings of the ATBC Study have been published elsewhere (ATBC Cancer Prevention Study Group, 1994; Albanes *et al.*, 1996; Heinonen *et al.*, 1998). In brief, the ATBC Study was a randomized, placebo-controlled prevention trial that assessed whether supplementation with alpha-tocopherol, beta-carotene, or both, reduced the incidence of lung and other cancers among men who smoked at least five cigarettes per day. At baseline, a fasting serum sample was collected from all subjects as well as medical, demographic and other information. Although the intervention ended in 1993, the trial participants continued to be followed as a cohort for cancer incidence and mortality through the national Finnish Cancer Registry and Register of Causes of Death, respectively. A nested case–control sample set was constructed among the 20 305 men who provided a whole blood sample at the end of the study from April 1992 through March 1993. Using incidence density sampling, 584 cases of primary prostate cancer (ICD-9 code 185; ICD-10 code C61) were chosen, and 584 matched controls were selected from the cohort who were alive and free of prostate cancer at the time their matched case was diagnosed with prostate cancer.

Controls were also individually matched to cases on age ($\pm$ 5 years), intervention group, study clinic, and date of blood draw ($\pm$ 30 days) in a 1 : 1 ratio.

SNP selection and genotyping analysis

The genotyping assays were conducted by the Core Genotype Facility (CGF), Center for Cancer Research, National Cancer Institute. Since each of the genes under study has numerous single nucleotide polymorphisms (SNPs), we identified specific SNPs that were representative of the genetic variation for each gene. In order to do this, haplotypes were inferred using the statistical program Phase Version 2.02 (Stephens *et al.*, 2001). The statistical method HtSNP (Stram *et al.*, 2003) was used to identify SNPs that are in strong linkage disequilibrium and representative haplotype-tagged SNPs (ht-SNP) designated using default program settings. The ht-SNPs were ascertained by analysis of the CGF reference samples (102 subjects). SNPs with $> 5\%$ allele frequency were identified and common haplotypes ($> 5\%$) estimated for each gene. The ht-SNP necessary to reconstruct the common haplotypes in white populations for each of the genes are as follows: IL-8 ($-251A \rightarrow T$), CXCR1 (exon 2, $+860C \rightarrow G$), and CXCR2 (untranslated 3′ region of exon 3, $-1010A \rightarrow G$).

The assays were performed using the Taqman platform (Applied Biosystems, Foster City, California, USA). The primer and probe sequences and conditions can be found at the CGF website http://snp500cancer.nci.nih.gov/home_1.cfm using the following reference IDs: IL-8, rs4073; CXCR1, rs2230054; and CXCR2, rs11226580.

We tested for quality control by repeating the assay for a sample of controls. The discordance rates for the sample was 4% (3/62), 0% (0/61), and 7% (4/58) for IL-8, CXCR1, and CXCR2, respectively. In some cases, genotyping assays did not work due to failure to amplify DNA or inadequate or poor-quality DNA. Therefore, genotyping data were available for 541 cases and 448 controls, the final sample used for this analysis. Controls that were not able to be genotyped did not differ statistically in terms of demographic, medical, or lifestyle characteristics from the controls included in the final statistical analysis ($P > 0.1$).

Data analysis

The chi-squared test was used to test the distribution of genotypes for the cases and controls. We further examined the association between the genotypes and prostate cancer risk by conditional logistic regression, and OR with the corresponding 95% CIs were calculated. The OR and 95% CI for the association between polymorphic IL-8 and receptor genes and clinico-pathologic characteristics among cases were estimated using unconditional logistic regression analysis. All reported OR and 95% CI for the polymorphisms are relative to the 'common' alleles, defined as the most frequent homozygote genotype. Additional exploratory analyses of gene–gene interactions between IL-8 and its receptors were

performed by stratification by IL-8. Covariates were included in the regression model if they changed the OR by > 20%. All statistical analyses were performed using STATA 7.0 (Stata Corporation, College Station, Texas, USA).

Results and discussion

In the present study, we assessed the association between prostate cancer risk among participants in the ATBC Cancer Prevention Study and the most representative allele for the IL-8 and its cytokine receptor genes: IL-8–251 (A→T), CXCR1 +860 (C→G), and CXCR2 −1010 (A→G) polymorphisms.

We did not observe statistically significant differences in the distribution of demographic, medical, and lifestyle characteristics including body mass index, smoke pack-years, and serum measurements of antioxidants between prostate cancer cases and matched controls. The cases were diagnosed with prostate cancer between 1986 and 1999 with a mean age at time of prostate cancer diagnosis of 68 ± 5 years, and an average survival time of 3 ± 3 years from prostate cancer diagnosis. The percentage of cases diagnosed with stages 0–I, stage II and stages III–IV were 31, 38 and 31%, respectively. Approximately 26, 51 and 23% of the cases were grade 1 (roughly equivalent to Gleason grades 1–4), grade 2 (Gleason grades 5–7) and grade 3 (Gleason grades 8–10) tumours, respectively.

Table 1 Association between polymorphisms of interleukin 8 (*IL-8*) and its receptors (*CXCR1* and *CXCR2*) and prostate cancer risk in the nested case–matched control[a] study of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study

	Cases (n=541)		Controls (n=448)		OR[b] (95% CI)
	n	%	n	%	
IL-8 (promoter −251)					
TT	181	33	135	30	1
AT	236	44	217	48	0.8 (0.6–1.1)
AA	103	19	66	15	1.2 (0.8–1.8)
Undetermined	21	4	30	7	
TT	181	35	135	32	1
AT and AA combined	339	65	283	68	0.9 (0.7–1.2)
CXCR1 (exon 2 +860)[c]					
GG	478	88	389	87	1
CG	53	10	41	9	1.0 (0.6–1.7)
CC	3	0	0		0 (0-.)
Undetermined	7	1	18	4	
GG	478	90	389	90	1
CG and CC combined	56	10	41	10	1.1 (0.7–1.7)
CXCR2 (exon 3 −1010)[d]					
GG	213	39	177	40	1
AG	226	42	191	43	0.9 (0.7–1.3)
AA	68	13	44	10	1.0 (0.6–1.7)
Undetermined	34	6	36	8	
GG	213	43	177	43	1
AG and AA combined	294	57	235	57	1.0 (0.7–1.3)

[a]Cases and controls are individually matched on 5 year age group, intervention group, study clinic, and date of blood draw (±30 days) in a 1 : 1 ratio.
[b]Odds ratios (ORs) adjusted for additional potential confounders (i.e. body mass index, pack years of smoking) were not significantly different from the unadjusted odds ratio estimates, thus, unadjusted matched odd ratios are reported. CI, confidence interval.
[c]Previously known as *IL-8RA*.
[d]Previously known as *IL-8RB*.

The genotype distribution of the controls for IL-8, CXCR1 and CXCR2 are in Hardy–Weinberg equilibrium ($P = 0.2$, $P = 0.2$, $P = 0.5$, respectively). As shown in Table 1, case–control analysis did not reveal statistically significant differences in the distribution of IL-8, CXCR1 and CXCR2 polymorphisms between prostate cancer cases and controls. We found that individuals of the AA genotype for IL-8 may be 20% more likely to develop prostate cancer than those individuals with TT genotypes, although this association failed to reach statistical significance. Our finding of an elevated odds of prostate cancer for those men with the AA genotype is in the same direction as that reported by McCarron *et al.* (2002), but the strength of the association we observed was weaker (OR 1.2, 95% CI 0.8–1.8). This inconsistency may be a result of sampling or inherent genetic differences between the Finnish and British populations studied. It is interesting to note that the genotype distribution for IL-8 among the controls in our Finnish population was significantly different from that of McCarron's British study population ($P = 0.04$).

Among prostate cases, there is no association between IL-8, CXCR1 and CXCR2 with clinical stage (TNM stage), tumour grade of disease (based on tumour differentiation), or the metastatic status of the prostate cancer. Tests of the linear trend for risk across tumour grade, stage, or metastatic profile were not significant ($P > 0.1$). Survival of patients with prostate cancer stratified by IL-8 showed a 40% reduction of dying from cancer among those carriers with the IL-8 genotype carriers with the A allele compared with IL-8 homozygous TT genotype (unadjusted OR 0.6, 95% CI 0.4–1.0). Although this finding is intriguing, this association could be observed purely by chance alone and would need to be replicated in other independent studies for strong inferences to be drawn.

Table 2 illustrates our exploratory analysis of the possible joint effect of IL-8 with each of the two receptors. There appears to be a differential prostate cancer risk among the receptor genotypes stratified by IL-8. Participants who had the variant alleles for IL-8 and CXCR1 had a statistically significant twofold increase in the risk of prostate cancer while those who were variant for IL-8 and CXCR2 had a non-significant 30% reduction in risk. It should be noted, however, that the confidence intervals of all four risk estimates overlapped and that the interaction term was not statistically significant.

While no functional studies have been performed to determine the phenotypic effects of the receptors or this interaction, this result is interesting given that we did not

Table 2 Association between combinations of polymorphisms of interleukin 8 *(IL-8)* and its receptors (*CXCR1* and *CXCR2*) and prostate cancer cases and matched[a] controls in the nested case–control study of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study

	IL-8 TT genotype carriers					IL-8 AT or AA genotype carriers				
	Case		Control		OR[b] (95% CI)	Case		Control		OR[b] (95% CI)
	n	%	*n*	%		*n*	%	*n*	%	
CXCR1										
GG	164	93	114	88	1	296	88	246	91	1
CG and CC	12	7	15	12	1.0 (0.1–7.1)	42	12	25	9	2 (1.0–4.1)
CXCR2										
GG	65	38	55	44	1	154	46	109	42	1
AG and AA	106	62	70	56	1.0 (0.4–2.9)	182	54	151	58	0.7 (0.4–1.2)

[a]Cases and controls are individually matched on 5 year age group, intervention group, study clinic, and date of blood draw (±30 days) in a 1:1 ratio.
[b]Odds ratios (ORs) adjusted for additional potential confounders (i.e. body mass index, pack-years of smoking) were not significantly different from the unadjusted OR estimates, thus, unadjusted matched ORs are reported. CI, confidence interval.

see any association between the receptors themselves and prostate cancer risk or prognosis. It is possible this difference in magnitude of prostate cancer risk between the receptors may be a function of IL-8 competition with other chemokines for the receptor. These two receptors are homologous over 78% of the amino acid and both bind to IL-8 with high affinity (Holmes *et al.*, 1991; Lee *et al.*, 1992). However, whereas CXCR1 binds only to IL-8 and GCP-2, CXCR2 binds to additional chemokine ligands such as GROα and NAP-2 (Moser *et al.*, 1991; Ahuja and Murphy, 1996; Rossi and Zlotnik, 2000). Therefore, in the presence of increased level of IL-8, it is possible that the difference in IL-8 binding affinity to two different IL-8 receptors may be functionally significant in prostate cancer risk.

In conclusion, our data indicate that germline variations in polymorphic IL-8 and its receptor genes alone may not play a major role in prostate cancer risk or prognosis. Although none of the SNPs studied are likely to have major effects on prostate cancer susceptibility, a role for other polymorphisms associated within these genes cannot be excluded. Future studies which further characterize and compile a comprehensive genetic cytokine profile may aid in the resolution of the role IL-8 plays in prostate cancer risk and disease progression.

References

Ahuja SK, Murphy PM (1996). The CXC chemokines growth-regulated oncogene (GRO) alpha, GRObeta, GROgamma, neutrophil-activating peptide-2, and epithelial cell-derived neutrophil-activating peptide-78 are potent agonists for the type B, but not the type A, human interleukin-8 receptor. *J Biol Chem* **271**:20545–20550.

Albanes D, Heinonen OP, Taylor PR, Virtamo J, Edwards BK, Rautalahti M, *et al.* (1996). Alpha-tocopherol and beta-carotene supplements and lung cancer incidence in the alpha-tocopherol, beta-carotene cancer prevention study: effects of base-line characteristics and study compliance. *J Natl Cancer Inst* **88**:1560–1570.

ATBC Cancer Prevention Study Group (1994). The alpha-tocopherol, beta-carotene lung cancer prevention study: design, methods, participant characteristics, and compliance. The ATBC Cancer Prevention Study Group. *Ann Epidemiol* **4**:1–9.

Balbay MD, Pettaway CA, Kuniyasu H, Inoue K, Ramirez E, Li E, *et al.* (1999). Highly metastatic human prostate cancer growing within the prostate of athymic mice overexpresses vascular endothelial growth factor. *Clin Cancer Res* **5**:783–789.

Coussens LM, Werb Z (2002). Inflammation and cancer. *Nature* **420**: 860–867.

Ferrer FA, Miller LJ, Andrawis RI, Kurtzman SH, Albertsen PC, Laudone VP, *et al.* (1998). Angiogenesis and prostate cancer: *in vivo* and *in vitro* expression of angiogenesis factors by prostate cancer cells. *Urology* **51**:161–167.

Greene GF, Kitadai Y, Pettaway CA, von Eschenbach AC, Bucana CD, Fidler IJ (1997). Correlation of metastasis-related gene expression with metastatic potential in human prostate carcinoma cells implanted in nude mice using an *in situ* messenger RNA hybridization technique. *Am J Pathol* **150**: 1571–1582.

Heinonen OP, Albanes D, Virtamo J, Taylor PR, Huttunen JK, Hartman AM, *et al.* (1998). Prostate cancer and supplementation with alpha-tocopherol and beta-carotene: incidence and mortality in a controlled trial. *J Natl Cancer Inst* **90**:440–446.

Holmes WE, Lee J, Kuang WJ, Rice GC, Wood WI (1991). Structure and functional expression of a human interleukin-8 receptor. *Science* **253**:1278–1280.

Hull J, Thomson A, Kwiatkowski D (2000). Association of respiratory syncytial virus bronchiolitis with the interleukin 8 gene region in UK families. *Thorax* **55**:1023–1027.

Inoue K, Slaton JW, Eve BY, Kim SJ, Perrotte P, Balbay MD, *et al.* (2000). Interleukin 8 expression regulates tumorigenicity and metastases in androgen-independent prostate cancer. *Clin Cancer Res* **6**:2104–2119.

Kim SJ, Uehara H, Karashima T, Mccarty M, Shih N, Fidler IJ (2001). Expression of interleukin-8 correlates with angiogenesis, tumorigenicity, and metastasis of human prostate cancer cells implanted orthotopically in nude mice. *Neoplasia* **3**:33–42.

Konig JE, Senge T, Allhoff EP, Konig W (2004). Analysis of the inflammatory network in benign prostate hyperplasia and prostate cancer. *Prostate* **58**:121–129.

Landi S, Moreno V, Gioia-Patricola L, Guino E, Navarro M, de Oca J, *et al.* (2003). Association of common polymorphisms in inflammatory genes interleukin (IL)6, IL8, tumor necrosis factor alpha, NFKB1, and peroxisome proliferator-activated receptor gamma with colorectal cancer. *Cancer Res* **63**: 3560–3566.

Lee J, Horuk R, Rice GC, Bennett GL, Camerato T, Wood WI (1992). Characterization of two high affinity human interleukin-8 receptors. *J Biol Chem* **267**:16283–16287.

McCarron SL, Edwards S, Evans PR, Gibbs R, Dearnaley DP, Dowe A, *et al.* (2002). Influence of cytokine gene polymorphisms on the development of prostate cancer. *Cancer Res* **62**:3369–3372.

Moore BB, Arenberg DA, Stoy K, Morgan T, Addison CL, Morris SB, *et al.* (1999). Distinct CXC chemokines mediate tumorigenicity of prostate cancer cells. *Am J Pathol* **154**:1503–1512.

Moser B, Schumacher C, von Tschamer V, Clark-Lewis I, Baggiolini M (1991). Neutrophil-activating peptide 2 and gro/melanoma growth-stimulatory activity interact with neutrophil-activating peptide 1/interleukin 8 receptors on human neutrophils. *J Biol Chem* **266**:10666–10671.

Reiland J, Furcht LT, McCarthy JB (1999). CXC-chemokines stimulate invasion and chemotaxis in prostate carcinoma cells through the CXCR2 receptor. *Prostate* **41**:78–88.

Rossi D, Zlotnik A (2000). The biology of chemokines and their receptors. *Annu Rev Immunol* **18**:217–242.

Savage SA, Abnet CC, Mark SD, Qiao YL, Dong ZW, Dawsey SM, *et al.* (2004). Variants of the IL8 and IL8RB genes and risk for gastric cardia adenocarcinoma and esophageal squamous cell carcinoma. *Cancer Epidemiol Biomarkers Prev* **13**:2251–2257.

Stephens M, Smith NJ, Donnelly P (2001). A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* **68**: 978–989.

Stram DO, Leigh-Pearce C, Bretsky P, Freedman M, Hirschhorn JN, Altshuler D, *et al.* (2003). Modeling and E-M estimation of haplotype-specific risks from genotype data for a case–control study of unrelated individuals. *Hum Hered* **55**:179–190.

van der Kuyl AC, Polstra AM, Weverling GJ, Zorgdrager F, van den Burg R, Cornelissen M (2004). An IL-8 gene promoter polymorphism is associated with the risk of the development of AIDS-related Kaposi's sarcoma: a case–control study. *AIDS* **18**:1206–1208.

Veltri RW, Miller MC, Zhao G, Ng A, Marley GM, Wright GL Jr, *et al.* (1999). Interleukin-8 serum levels in patients with benign prostatic hyperplasia and prostate cancer. *Urology* **53**:139–147.

Xie K (2001). Interleukin-8 and human cancer biology. *Cytokine Growth Factor Rev* **12**:375–391.